UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

July 20, 2004

**MEMORANDUM:**

**Subject:** Efficacy Review for EPA Reg. No.: 9480-4, Sani-Cloth® Germicidal Wipes; DP Barcode 301189

**From:** Tajah Blackburn, Ph.D., Microbiologist
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**Thru:** Nancy Whyte, Acting Team Leader
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**To:** Marshall Swindell PM 33/ Tony Kish
Regulatory Branch I
Antimicrobials Division (7510C)

**Applicant:** PDI, The Healthcare Division of Nice-Pak Products, Inc.

Two Nice-Pak Park
Orangeburg, NY 10962-1376

**Formulation from Label:**

| Active ingredient(s) | % by wt. |
|---|---|
| n-Alkyl (68% $C_{12}$, 32% $C_{14}$) dimethyl ethylbenzyl ammonium chlorides ..... | 0.25% |
| n-Alkyl (60% $C_{14}$, 30% $C_{16}$, 5% $C_{12}$, 5% $C_{18}$) dimethyl benzyl ammonium chlorides ..... | 0.25% |
| Isopropyl Alcohol ..... | 55.00% |
| Other ingredients ..... | 44.50% |
| Total ..... | 100.00% |

## I BACKGROUND

The product, Sani-Cloth® Germicidal Disposable Wipes (EPA Reg. No. 9480-4), is an EPA -registered disinfectant (bactericide and virucide). The registrant referenced a letter faxed from the Agency on 2/26/03, permitting the use of the expressed liquid from the towelette for efficacy studies against HBV. Therefore, the submitted HBV efficacy studies were conducted using expressed liquid from the towelette Sani-Cloth® Germicidal Disposable Wipes (EPA Reg. No. 9480-4), in accordance with the Agency's letter. Additionally the applicant requested an amendment to the registration of this product to add claims for Human Respiratory Syncytial virus. The applicant has also indicated that the product name in the study is listed as Super Sani-Cloth, an alternate brand name for the product Sani-Cloth Germicidal Wipes. Efficacy studies were conducted at ATS Labs located at 2540 Executive Drive, St. Paul, MN 55120.

This data package contained letter from the applicant, faxed response from the Agency, EPA Form 8570-4 (Confidential Statement of Formula), two studies (MRID No. 462331-01 and 462331-02), Statements of No Data Confidentiality for both data packages, and the proposed label.

## II USE DIRECTIONS

This towelette product is designed for use on hard, non-porous surfaces such as toilet seats, bathroom fixtures, vanity tops, shower stalls, bathtubs, tiles, sinks, doorknobs, telephones, laundry rooms, garbage cans, pet areas, dictating equipment surfaces, computer keyboards/mouse, scales, televisions, changing tables, cribs, diaper pails, trash cans, toys, stretchers, stethoscopes, and the external surfaces of dialysis machines located in bathrooms, nurseries, daycare facilities, and hospital/healthcare settings, respectively.

For disinfection: Use a wipe to remove gross filth. Unfold a clean wipe and thoroughly wet surfaces. Treated surfaces must remain visibly wet for a full five (5) minutes. Use additional wipe(s) if needed to assure continuous five (5) minute wet contact time. Let air dry.

For virucidal and tuberculocidal activity: Use a wipe to remove gross filth. Unfold a clean wipe and thoroughly wet surface. Treated surfaces must remain visibly wet for one (1) minute. Let air dry. For food contact surfaces, follow one (1) minute contact time with a thorough potable water rinse. Although efficacy at a one (1) minute contact time has been shown to be adequate against the specific organisms listed above, this time is not sufficient for other organisms listed on the label. Therefore a 5 minute wet contact time must be used for all other organisms.

Note– The proposed label directions indicate that: "This product is not to be used as a terminal sterilant/high level disinfectant on any surface or instrument that. . . ."

## III AGENCY STANDARDS FOR PROPOSED CLAIMS

Disinfectants for Use on Hard Surfaces Using Pre-Saturated or Impregnated Towelettes

Towelette products represent a unique combination of antimicrobial chemical and applicator, pre-packaged as a unit in fixed proportions. As such, the complete product, as offered for sale, should be tested according to the directions for use to ensure the product's effectiveness in disinfecting hard surfaces. The standard test methods available for hard surface disinfectants (i.e., AOAC Use-Dilution Method, AOAC Germicidal Spray Products test), if followed exactly, would not closely simulate the way a towelette product is used. Agency guidelines recommend that a simulated-use test be conducted by modifying the AOAC Germicidal Spray Products Test. Agency guidelines further recommend that instead of spraying the inoculated surfaces of the glass slide, the product should be tested by wiping the surface of the glass slide with the saturated towelette, and then subculturing the slides after a specified holding time. Liquid expressed from the used towelette should also be subcultured. Sixty carriers must be tested with each of 3 product samples, representing 3 different product batches, one of which is at least 60 days old. To support products labeled as "disinfectants," killing on 59 put of 60 carriers (for both the slide subculture and the expressed liquid from the towelette) is required to provide effectiveness at the 95% confidence level. The above Agency standards are presented in DIS/TSS-1 and EPA Pesticide Assessment Guidelines, Subdivision G, § 91-2(h), Pre-saturated or Impregnated Towelettes.

Effectiveness of disinfectants against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, but not including viruses, may also be determined by the modified version of the AOAC Germicidal Spray Products Test. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different batches. To support products labeled as "disinfectants" for specific microorganisms (other than those microorganisms named in the above test method), killing of the specific microorganism on all carriers (for both the slide subculture and the expressed liquid from the towelette) is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least $10^4$ microorganisms survived the carrier-drying step.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products Test (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be tested must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least $10^4$ from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique. The calculated viral titers must be reported with the test results. For data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond cytotoxic level.

## IV COMMENTS ON SUBMITTED EFFICACY STUDIES

**1. MRID No. 462331-01, "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B" (A Surrogate for Human Hepatitis B) by Karen M. Ramm, B.A. Study conducted at ATS Labs. Study completion date– March 11, 2004.**

The product Super Sani Cloth was tested for efficacy against Duck Hepatitis B Virus-Strain DHBV 16 ( obtained from Hepadnavirus Testing Palo Alto, CA) using primary duck hepatocytes (obtained from purchased ducklings) as the host system. The study protocol

followed ATS Protocol "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B" dated March 11, 2004. Two lots (Lot Nos. 3B001STY and 3B059STY) were tested in the presence of 100% duck serum. Liquid was aseptically expressed from Sani-Cloth® Plus towelettes and used undiluted as requested by the Sponsor. The expressed liquid was in solution as determined by visual observations and used on the day it was expressed from the towelettes. Films of virus were prepared at staggered intervals by spreading 0.2 mL of virus inoculum uniformly over the bottoms of ten separate glass carriers. The virus films were air-dried at 20.0°C at a relative humidity of 22-23% for 30 minutes. Carriers were exposed to 2.0 mL of the use dilution for 2 minutes at 20°C. Following the exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-disinfectant mixture, for subsequent passage through a Sephadex column for neutralization. The filtrate was then titered by serial dilution. Following titration, the $10^{-2}$ and $10^{-3}$ dilutions of each replicate of the test substance were passed through individual Sephadex column utilizing the syringe plunger to aid in reducing the cytotoxic effects of the test substance. Primary duck hepatocytes were inoculated in quadruplicate with 1.0 mL of the dilutions and incubated at 36-38°C in 5-7% $CO_2$ for 10 days. The test medium was aspirated from each well and replaced with fresh medium as needed throughout the incubation period. On the final day of incubation, the cultures were scored for cytotoxicity and the cells were fixed with ethanol. An indirect immunofluorescence assay was performed using a monoclonal antibody specific for the envelope protein of the virus, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. BTC 835 (EPA Reg. No. 1839-32) was used as the data consistency control at two concentrations, 175 ppm and 350 ppm.

Note– In the submitted letter included in the data package, the applicant stated that the enclosed studies listed Super Sani Cloth as the test system, and that Sani-Cloth Germicidal Wipes and Super Sani Cloth are synonymous. However in the efficacy study (MRID No. 462331-01), item #1 in the Test Method section (page 9 of 34), the liquid expressed from towelette originates from Sani-Cloth Plus towelettes (MRID No. 462333-01), not from Super Sani Cloth as mentioned (MRID No. 462331-01, page 8 of 34).

**2. MRID No. 462331-01, "Virucidal Efficacy of a Disinfectant for Use on Inanimate**

**Environmental Surfaces Utilizing Duck Hepatitis B" (A Surrogate for Human Hepatitis B)Confirmatory Assay by Mary J. Miller, M.T. Study conducted at ATS Labs. Study completion date– December 23, 2004.**

The product Super Sani Cloth was tested for efficacy against Duck Hepatitis B Virus-Strain DHBV 16 ( obtained from Hepadnavirus Testing Palo Alto, CA) using primary duck hepatocytes (obtained from purchased ducklings) as the host system. The study protocol followed ATS Protocol "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces Utilizing Duck Hepatitis B" dated December 23, 2003. One lot (Lot No. 3B001STY) was tested in the presence of 100% duck serum. Liquid was aseptically expressed from Super Sani Cloth towelettes and used undiluted as requested by the Sponsor. The expressed liquid was in solution as determined by visual observations and used on the day it was expressed from the towelettes. Films of virus were prepared at staggered intervals by spreading 0.2 mL of virus inoculum uniformly over the bottoms of eight separate glass carriers. The virus films were air-dried at 20.0°C at a relative humidity of 41% for 30 minutes. Carriers were exposed to 2.0 mL of the use dilution for 2 minutes at 20°C. Following the exposure time, the plates were scraped with a cell scraper to resuspend the contents of the plate and the virus-disinfectant mixture, for subsequent passage through a Sephadex column for neutralization. The filtrate was titered by serial dilution. Following titration, the $10^{-2}$ and $10^{-3}$ dilutions of each replicate of the test substance were passed through individual Sephadex column utilizing the syringe plunger to aid in reducing the cytotoxic effects of the test substance. Primary duck hepatocytes were inoculated in quadruplicate with 1.0 mL of the dilutions and incubated at 36-38°C in 5-7% $CO_2$ for 10 days. The test medium was aspirated from each well and replaced with fresh medium as needed throughout the incubation period. On the final day of incubation, the cultures were scored for cytotoxicity and the cells were fixed with ethanol. An indirect immunofluorescence assay was performed using a monoclonal antibody specific for the envelope protein of the virus, and enumerated as Most Probable Number (MPN). Controls included cell viability, virus stock titer, plate recovery, column titer, neutralizer effectiveness, cytotoxicity, and data consistency. BTC 835 (EPA Reg. No. 1839-32) was used as the data consistency control at two concentrations, 175 ppm and 350 ppm.

**Note**– Due to the "unavailability" of Swimm's S77 medium for the repeat assay, Williams's

Medium E containing collagenase was used for *in situ* perfusion of the duck liver.

**Note**– Per lab report, this [study] was repeated to obtain recoverable dried virus control titers of $\geq 10^4$ from the test surface and to demonstrate a ≥3 log reduction in titer beyond the cytotoxic level.

**3. MRID No. 462331-02, "Virucidal Efficacy of Pre-Saturated Towelettes for Hard Surface Disinfection Using Respiratory Syncytial Virus," by Mary Miller, M.T. Study conducted at ATS Labs. Study completion date– November 5, 2003.**

The study was conducted using Respiratory Syncytial Virus- Long strain (ATCC VR-26) with Hep-2 cells (obtained ViroMed Laboratories, Inc.) as the host system. Two lots (Lot Nos. 3B001STY and 3B059STY) of the product Super Sani Cloth Cloths were tested according to an ATS Labs protocol (Protocol No. NPP01082003.RSV; copy not provided). The test product was received undiluted. For the control system, a clean, unsaturated towelette was prepared by adding 3.7 g of sterile deionized water to one towelette. The stock virus contained 5% fetal bovine serum. Films of the virus were prepared by spreading 0.2 mL of inoculum uniformly over the bottoms of four separate sterile glass Petri dishes. The virus films were dried at 20.0°C at a relative humidity of 42% for 20 minutes, until visibly dry. Two dried virus films, as previously described, were used for each lot of test substance. Using sterile gloves, the dried virus film on the surface of the glass petri dish was wiped with a saturated towelette for ten seconds, and held at room temperature (22.5°C) for the remainder of the one minute exposure period. Immediately following the exposure period, a 2.0 mL aliquot of test medium was added to the petri dish, the plate was scraped with a plastic cell scraper to resuspend the contents of the plate, and subsequently passed through a Sephadex column. Test medium was added to the expressed liquid from the used towelette, and passage through a Sephadex column. Both filtrates were then titered by serial dilution, and the dilutions were assayed for infectivity and/or cytotoxicity. Hep-2 cells in multiwell culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. Cultures were incubated at 36-38°C in 5-7% $CO_2$, and scored periodically for the 10-day incubation period for the presence or absence of CPE, cytotoxicity, and viability. Controls included cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity

titers were calculated by the method of Spearman Karber.

## V RESULTS

| MRID Number | Organism | Results | | | | | Dried Virus Control ($TCID_{50}$/0.1 mL) |
|---|---|---|---|---|---|---|---|
| 462331-01 | Duck Hepatitis B virus | | Lot No. 3B001STY (#1) | Lot No. 3B001STY (#2) | Lot No. 3B059STY (#1) | Lot No. 3B059STY (#2) | $10^{5.0}$ (#1)<br>$10^{5.25}$ (#2) |
| | | | No cytotoxicity | | | | |
| | | $10^{-2}$ to $10^{-4}$ dilutions | Complete inactivation | | | | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{1.5}$ | | | | |
| | | Log reduction | ≥3.55 | | | | |

| MRID No | Organism | Results | | | Dried Virus Control ($TCID_{50}$/0.1 mL) |
|---|---|---|---|---|---|
| 462331-01 | Duck Hepatitis B virus | | Lot No. 3B001STY (#1) | Lot No. 3B001STY (#2) | $10^{4.75}$ (#1)<br>$10^{4.5}$ (#2) |
| | | | No cytotoxicity | | |
| | | $10^{-2}$ to $10^{-4}$ dilutions | Complete inactivation | | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{1.5}$ | | |
| | | Log reduction | ≥3.09 | | |

| MRID No. | Organism | Results | | | | | Dried Virus Control ($TCID_{50}$/0.1 mL) |
|---|---|---|---|---|---|---|---|
| | | | Lot No. 3B001STY (Carrier) | Lot No. 3B001STY (Expressed) | Lot No. 3B059STY (Carrier) | Lot No. 3B059STY (Expressed) | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 462331–02 | Respiratory Syncytial Virus | Cytotoxicity | None | $10^{-1}$ | None | $10^{-1}$ | $*10^{4.5}$ |
| | | $10^{-2}$ to $10^{-6}$ dilutions | Complete Inactivation | Complete Inactivation | Complete inactivation | Complete Inactivation | |
| | | $TCID_{50}$/0.1 mL | $\leq 10^{0.5}$ | $\leq 10^{1.5}$ | $\leq 10^{0.5}$ | $\leq 10^{1.5}$ | ** $10^{1.5}$ (carrier) |
| | | Log reduction | * 4 log reduction **1 log reduction | * 3 log reduction *** 1 log reduction | * 4 log reduction **1 log reduction | * 3 log reduction *** 1 log reduction | ***$10^{2.5}$ (expressed) |

Note– The control data [Table 1 (MRID No. 462333-02)] imply that there is a log reduction on the "carrier surface" (3 log reduction) and "expressed towelette" (3 log reduction). Based on the experimental data, there is a discrepancy in the "true" log reduction of the test system, (i.e., "carrier surface" (1 log reduction) and "expressed towelette" (1 log reduction).

Note– Cytotoxicity was observed in the $10^{-1}$ dilution using the control expressed towelette.

## VI CONCLUSIONS

1. The submitted efficacy data (MRID No. 462331-01) support the use of the product, Super Sani Cloths as a disinfectant with virucidal activity when tested against Duck Hepatitis B Virus in the presence of 100% organic soil load on hard, non-porous surfaces for a contact time of 2 minutes. A letter from the Agency permitted the use of the expressed liquid from the towelette for efficacy testing. Although the data was acceptable, critical and relevant information was missing from the study protocol, such as: (1) the number of towelettes used to obtain the said amount of liquid (2.0 mL), and (2) possible pooling of the liquid expressed from numerous towelettes. Furthermore in the submitted study designated MRID 462331–01, in the section Test Method (item #1; page 9 of 34), the liquid was expressed from Sani-Cloth Plus towelettes, not Super Sani Cloths, as previously mentioned. Please clarify and submit appropriate corrections.

2. The submitted efficacy data (MRID No. 462331-02) support the use of the product, Super Sani Cloths as a disinfectant with virucidal activity when tested against Respiratory Syncytial Virus (RSV) in the presence of 5% organic soil on hard, non-porous surfaces for a contact time of 1 minute. In the absence of the active ingredients, the control data, in Table 1, demonstrated a significant reduction in viral titer. When comparing the two data sets, the actual log reduction of the test system is unclear. However, the log reduction observed with the test system is sufficient to render the product efficacious.

## VII RECOMMENDATIONS

1. The proposed label claims (MRID No. 462331-01) are acceptable regarding the use of the product, Sani-Cloth® Germicidal Disposable Cloth as a disinfectant with virucidal activity against Duck Hepatitis B Virus in the presence of 100% organic soil on hard, non-porous surfaces for a contact time of at least 2 minutes.

2. The proposed label claims (MRID No. 462331-02) are acceptable regarding the use of the product, Sani-Cloth® Germicidal Disposable Cloth as a disinfectant with virucidal activity against Respiratory Syncytial Virus in the presence of 5% organic soil on hard, non-porous surfaces for a contact time of 1 minute. Log reduction discrepancies, between control data and the test system, have been noted.